



**DECLARATION UNDER 37 C.F.R § 1.132**

Sir:

I, Wayne Comper, hereby declare and say as follows:

1. That I am an Australian citizen , residing in Victoria, Australia, and am the inventor of the invention described and claimed in application Serial No. 09/892,797.

My curriculum vitae is attached as Exhibit A.

2. I have read and am familiar with the disclosure and pending claims of the above-captioned Application, as well as the issues raised in the pending final Office Action dated May 31, 2002.

3. I declare further that the following experiments were conducted at my direction and under my supervision and that the test results are true and correct to the best of my knowledge.

Two groups of rats were studied: I) untreated controls and ii) rats with experimental diabetes induced by administration of streptozotocin (STZ). Each of the rats was implanted with an osmotic pump containing radiolabelled IgG or transferrin. Steady state levels of radiolabelled protein in plasma were observed seven days after implantation of the osmotic pump. At that time collections were made of both urine and plasma. Estimates of the specific activity of protein in plasma were made by analysis of radioactivity and immuno-reactive material detected in the plasma. Immuno-unreactive (ghost) material in urine was estimated by the amount of radioactivity eluting at the position of the intact protein on a size exclusion column and specific activity of the protein in plasma.

The results for transferrin are set forth in the bar graph attached hereto. The amount of transferrin detected by immunoassay in both control animals and diabetic animals is about six times less than the amount of ghost transferrin detected. The amount of ghost transferrin estimated to be present in the urine of diabetic mice was significantly higher than in control rats, i.e., about a 47% increase in diabetic rats was observed. In contrast, the amount of intact transferrin detected by conventional radioimmune assay in the diabetic animals was not significantly different from that observed in the control animals.

The results obtained for IgG were similar to that of transferrin in that the amount of intact IgG detected by conventional radioimmune assay in control and diabetic animals was about the same. However, in both sets of animals, the amount of immuno-unreactive (ghost) material was significantly higher than the amount of intact IgG (at least about six times higher). The amount of ghost IgG was not significantly different in control and diabetic rats indicating that changes in IgG content in urine probably occur later than that observed for transferrin in diabetic animals.

### Conclusion

In my opinion, the test results demonstrate that:

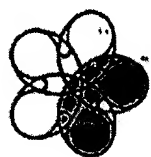
- (1) The majority of protein present in urine is not detectable by conventional immunoassay. As a result, conventional immunoassays are insufficient to detect renal complications of disease prior to the onset of kidney degeneration.

(2) Detection of immunoreactive and immuno-unreactive protein in a sample provides an accurate protein profile of the sample, which can be used to accurately diagnose renal complications of disease before the onset of kidney degeneration.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

\_\_\_\_\_  
Date

\_\_\_\_\_  
Wayne Comper



# National Kidney Foundation

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## Diabetes and Kidney Disease

Diabetes mellitus, usually called diabetes, is a disease in which your body does not make enough insulin or cannot use normal amounts of insulin properly. Insulin is a hormone that regulates the amount of sugar in your blood. A high blood sugar level can cause problems in many parts of your body.

## **Are there different types of diabetes?**

The most common ones are Type 1 and Type 2. Type 1 diabetes usually occurs in children. It is also called juvenile onset diabetes mellitus or insulin-dependent diabetes mellitus. In this type, your pancreas does not make enough insulin and you have to take insulin injections for the rest of your life.

Type 2 diabetes, which is more common, usually occurs in people over 40 and is called adult onset diabetes mellitus. It is also called non insulin-dependent diabetes mellitus. In Type 2, your pancreas makes insulin, but your body does not use it properly. The high blood sugar level often can be controlled by following a diet and/or taking medication, although some patients must take insulin. Type 2 diabetes is particularly prevalent among African Americans, American Indians, Latin Americans and Asian Americans.

## **What does diabetes do to the kidneys?**

With diabetes, the small blood vessels in the body are injured. When the blood vessels in the kidneys are injured, your kidneys cannot clean your blood properly. Your body will retain more water and salt than it should, which can result in weight gain and ankle swelling. You may have protein in your urine. Also, waste materials will build up in your blood.

Diabetes also may cause damage to nerves in your body. This can cause difficulty in emptying your bladder. The pressure resulting from your full bladder can back up and injure the kidneys. Also, if urine remains in your bladder for a long time, you can develop an infection from the rapid growth of bacteria in urine that has a high sugar level.

## **How many diabetic patients will develop kidney disease?**

About 30 percent of patients with Type 1 (juvenile onset) diabetes and 10 to 40 percent of those with Type 2 (adult onset) diabetes eventually will suffer from kidney failure.

## **What are the early signs of kidney disease in patients with diabetes?**

The earliest sign of diabetic kidney disease is an increased excretion of albumin in the urine. This is present long before the usual tests done in your doctor's office show evidence of kidney disease, so it is important for you to have this test on a yearly basis. Weight gain and ankle swelling may occur. You will use the bathroom more at night. Your blood pressure may get too high. As a person with diabetes, you should have your blood, urine and blood pressure checked at least once a year. This will lead to better control of your disease and early treatment of high blood pressure and kidney disease. Maintaining control of your diabetes can lower your risk of developing severe kidney disease.

## **What are the late signs of kidney disease in patients with diabetes?**

As your kidneys fail, your blood urea nitrogen (BUN) levels will rise as well as the level of creatinine in your blood. You may also experience nausea, vomiting, a loss of appetite, weakness, increasing fatigue, itching, muscle cramps (especially in your legs) and anemia (a low blood count). You may find you need less insulin. This is because diseased kidneys cause less breakdown of insulin. If you develop any of these signs, call your doctor.

## **TABLE 1**

### **Signs of Kidney Disease in Patients with Diabetes**

1. Albumin/protein in the urine
2. High blood pressure
3. Ankle and leg swelling, leg cramps
4. Going to the bathroom more often at night
5. High levels of BUN and creatinine in blood
6. Less need for insulin or antidiabetic medications
7. Morning sickness, nausea and vomiting
8. Weakness, paleness and anemia
9. Itching

## **What will happen if my kidneys have been damaged?**

First, the doctor needs to find out if your diabetes has caused the injury. Other diseases can cause kidney damage. Your kidneys will work better and last longer if you:

- Control your diabetes
- Control high blood pressure
- Get treatment for urinary tract infections
- Correct any problems in your urinary system
- Avoid any medicines that may damage the kidneys (especially over-the-counter pain medications)

If no other problems are found, your doctor will try to keep your kidneys working as long as possible. The use of high blood pressure medicines called angiotensin converting enzyme (ACE) inhibitors has been shown to help slow the loss of kidney function.

## **How are the kidneys kept working as long as possible?**

The kidney doctor, called a nephrologist, will plan your treatment with you, your family and your dietitian. Two things to keep in mind for keeping your kidneys healthy are controlling high blood pressure in conjunction with an ACE inhibitor and following your renal diabetic diet. Restricting protein in your diet also might be helpful. You and your dietitian can plan your diet together.

## **What is end stage renal failure in patients with diabetes?**

End stage renal failure, or kidney failure, occurs when your kidneys are no longer able to support you in a reasonably healthy state, and dialysis or transplantation is needed. This happens when your kidneys function at only 10 to 15 percent. The usual span of time between the onset of diabetic kidney injury and kidney failure is about five to seven years.

## How is kidney failure treated in diabetic patients?

Three types of treatment can be used once your kidneys have failed: kidney transplantation, hemodialysis and peritoneal dialysis.

## Can a patient with diabetes have a kidney transplant?

Yes. Once you get a new kidney, you may need a higher dose of insulin. Your appetite will improve so your new kidney will break down insulin better than your injured one. You will use steroids to keep your body from rejecting your new kidney. If your new kidney fails, dialysis treatment can be started while you wait for another kidney.

## What about pancreas transplants?

Sometimes it is possible to perform a pancreas transplant along with a kidney transplant. Your doctor can advise you about this possibility.

## What about a low-protein diet?

Research suggests that a low-protein diet can slow the advance of kidney damage.

## What is the future outlook for patients with diabetes?

Today, more and more research dollars are spent on diabetes research. Hopefully, the prevention and cure of diabetes is in our future. In the meantime, you can manage your diabetes better with:

- home monitoring of your blood glucose levels
- maintaining an awareness of controlling your blood pressure, and possibly monitoring your pressure at home
- following your special diet.

Updated: 05/18/05

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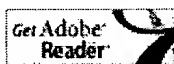
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### Tests

Tests performed on samples of your blood and urine are the first line of defense in detecting kidney problems and minimizing damage. Tests of these types can show how well the kidneys are removing excess fluids and waste. When a structural problem is suspected, a variety of imaging tests can be used to evaluate the kidney. A sample of kidney tissue, a biopsy, is sometimes helpful in diagnosing the specific cause of the problem.

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### Tests commonly used for screening and diagnosis

There are several tests commonly used to help your health care provider recognize if you have kidney or urinary disease. A blood sample can be analyzed for creatinine (and estimated glomerular filtration rate (EGFR)) and blood urea nitrogen (BUN). The level of these waste products in the blood increases as kidney filtration declines. Abnormal results are usually the first sign that kidney disease is present. A urine sample is typically also examined and analyzed (urinalysis) as part of routine screening. This set of tests looks for indicators of kidney and urinary disease such as red blood cells, white blood cells (WBCs or leukocytes), and protein in the urine. If you have diabetes or high blood pressure, the amount of protein (microalbumin) in your urine should be checked at least yearly to detect kidney disease in its early stages. When you have symptoms suggesting infection, a urine culture can confirm the presence of a bacterial infection.

### Tests to monitor kidney function

If you have been diagnosed with a kidney disease, your health care provider will order laboratory tests to help monitor kidney function. Blood levels of BUN and creatinine are measured from time to time to see if the kidney disease is getting worse. The amount of calcium and phosphate in the blood and the balance of serum and urine electrolytes can also be measured, as these are often affected by kidney disease. Hemoglobin, measured as part of a complete blood count (CBC) may be measured (the kidneys make a hormone, erythropoietin, that controls red blood cell production). Urine total protein can be used to test the effects of treatment in diabetes and nephrotic syndrome. Parathyroid hormone, which controls calcium levels, is often increased in kidney disease and is often checked to see if enough calcium and vitamin D are being taken to prevent bone damage.

Condition	Tests Used in Diagnosis	Tests Used to Follow
Chronic renal failure	BUN, creatinine, estimated GFR, urinalysis	BUN, creatinine, estimated GFR, electrolytes, calcium, phosphate, alkaline phosphatase, parathyroid hormone, CBC

Urinary tract infections	Urinalysis, urine culture	Urinalysis, urine culture
Kidney stones	Imaging, urinalysis	Urine sodium, calcium, phosphate, citrate, oxalate, uric acid
Nephrotic syndrome	Urinalysis, serum albumin, total protein, cholesterol, urine total protein, antinuclear antibody (ANA) test, hepatitis B test, hepatitis C test, complement levels	Urine total protein, serum cholesterol, BUN, creatinine, estimated GFR
Nephritic syndrome	Urinalysis, BUN, creatinine, estimated GFR, serum albumin, urine total protein, antinuclear antibody (ANA) test, antistreptolysin O, antiglomerular basement membrane antibody, antineutrophil cytoplasmic antibodies	BUN, creatinine, estimated GFR, urinalysis
Kidney disease due to diabetes or high blood pressure	Microalbumin	Microalbumin, urine total protein, BUN, creatinine, estimated GFR

#### *Imaging techniques*

If a structural problem or blockage is suspected, a picture of the kidneys can be helpful. Imaging techniques such as an ultrasound, CT scan (computed tomography), isotope scan, or intravenous pyelogram (IVP) may be used. Various x-ray procedures can also be employed, such as an intravenous urogram, micturating cystogram, or renal arteriogram (or angiogram).

#### *Kidney biopsy*

A biopsy can determine the cause of protein or blood in the urine and be used to monitor the effectiveness of treatment. Analyzing a small piece of kidney tissue can reveal the nature and extent of structural damage to a kidney. A biopsy, obtained using a biopsy needle and diagnostic imaging equipment, is often useful when disease of the glomerular filter is suspected.

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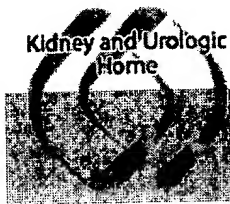
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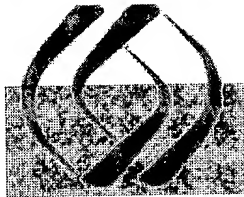
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## Proteinuria

### On this page:

- Who is at risk?
- What are the signs of proteinuria and kidney failure?
- What are the tests for proteinuria?
- How is proteinuria treated?
- Hope Through Research
- Points to Remember
- For More Information

Proteinuria describes a condition in which urine contains an abnormal amount of protein. Proteins are the building blocks for all body parts, including muscles, bones, hair, and nails. Proteins in your blood also perform a number of important functions. They protect you from infection, help your blood clot, and keep the right amount of fluid circulating throughout your body.

As blood passes through healthy kidneys, they filter the waste products out and leave in the things the body needs, like proteins. Most proteins are too big to pass through the kidneys' filters into the urine unless the kidneys are damaged. The main protein that is most likely to appear in urine is albumin. Proteins from the blood can escape into the urine when the filters of the kidney, called glomeruli, are damaged. Sometimes the term albuminuria is used when a urine test detects albumin specifically. Albumin's function in the body includes retention of fluid in the blood. It acts like a sponge, soaking up fluid from body tissues.

Inflammation in the glomeruli is called **glomerulonephritis**, or simply **nephritis**. Many diseases can cause this inflammation, which leads to proteinuria. Additional processes that can damage the glomeruli and cause proteinuria include diabetes, hypertension, and other forms of kidney diseases.

Research shows that the level and type of proteinuria (whether the urinary proteins are albumin only or include other proteins) strongly determine the extent of damage

and whether you are at risk for developing progressive kidney failure.

Proteinuria is also associated with cardiovascular disease. Damaged blood vessels may lead to heart failure or stroke as well as kidney failure. If your doctor finds that you have proteinuria, do what you can to protect your health and prevent any of these diseases from developing.

Several health organizations recommend that some people be regularly checked for proteinuria so that kidney disease can be detected and treated before it progresses. A 1996 study sponsored by the National Institutes of Health determined that proteinuria is the best predictor of progressive kidney failure in people with type 2 diabetes. The American Diabetes Association recommends regular urine testing for proteinuria for people with type 1 or type 2 diabetes. The National Kidney Foundation recommends that routine checkups include testing for excess protein in the urine, especially for people in high-risk groups.

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### Who is at risk?

People with diabetes, hypertension, or certain family backgrounds are at risk for proteinuria. In the United States, diabetes is the leading cause of end-stage renal disease (ESRD), the result of chronic kidney disease. In both type 1 and type 2 diabetes, the first sign of deteriorating kidney function is the presence of small amounts of albumin in the urine, a condition called microalbuminuria. As kidney function declines, the amount of albumin in the urine increases, and microalbuminuria becomes full-fledged proteinuria.

High blood pressure is the second leading cause of ESRD. Proteinuria in a person with high blood pressure is an indicator of declining kidney function. If the hypertension is not controlled, the person can progress to full renal failure.

African Americans are more likely than Caucasians to have high blood pressure and to develop kidney problems from it, even when their blood pressure is only mildly elevated. In fact, African Americans are six times more likely than Caucasians to develop hypertension-related kidney failure.

Other groups at risk for proteinuria are American Indians, Hispanic/Latinos, Pacific Islander Americans, older people, and overweight people. These at-risk groups and people who have a family history of kidney disease should have their urine tested regularly.

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### What are the signs of proteinuria and kidney failure?

Large amounts of protein in your urine may cause it to look foamy in the toilet. Also, because the protein has left your body, your blood can no longer soak up enough fluid and you may notice swelling in your hands, feet, abdomen, or face. These are

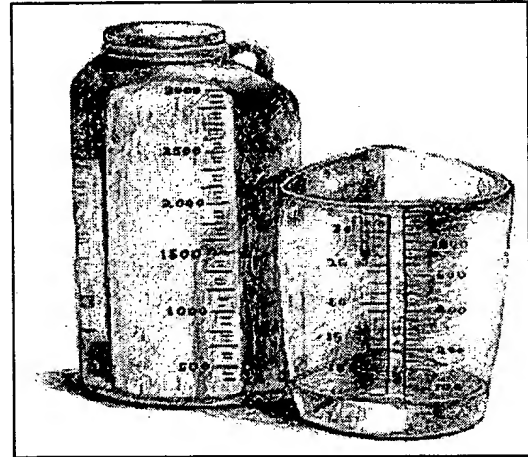
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signs of very large protein loss. More commonly, you may have proteinuria without noticing **any** signs or symptoms. Testing is the only way to find out how much protein you have in your urine.

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### What are the tests for proteinuria?

To test for proteinuria, you will need to give a urine sample. A strip of chemically treated paper will change color when dipped in urine that has too much protein. Laboratory tests that measure exact amounts of protein or albumin in the urine are recommended for people at risk for kidney disease, especially those with diabetes. The protein-to-creatinine or albumin-to-creatinine ratio can be measured on a sample of urine to detect smaller amounts of protein, which can indicate kidney disease. If the laboratory test shows high levels of protein, another test should be done 1 to 2 weeks later. If the second test also shows high levels of protein, you have persistent proteinuria and should have additional tests to evaluate your kidney function.



Containers for collecting urine.

Your doctor will also test a sample of your blood for creatinine and urea nitrogen. These are waste products that healthy kidneys remove from the blood. High levels of creatinine and urea nitrogen in your blood indicate that kidney function is impaired.

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### How is proteinuria treated?

If you have diabetes, hypertension, or both, the first goal of treatment will be to control your blood glucose and blood pressure. If you have diabetes, you should test your blood glucose often, follow a healthy eating plan, take your medicines, and get plenty of exercise. If you have diabetes and high blood pressure, your doctor may prescribe a medicine from a class of drugs called ACE (angiotensin-converting enzyme) inhibitors or a similar class called ARBs (angiotensin receptor blockers). These drugs have been found to protect kidney function even more than other drugs that provide the same level of blood pressure control. The American Diabetes Association recommends that people with diabetes keep their blood pressure below 130/80.

People who have high blood pressure and proteinuria but not diabetes also benefit from taking an ACE inhibitor or ARB. Their blood pressure should be maintained below 130/80. To maintain this target, you may need to take a combination of two or more blood pressure medicines. Your doctor may also prescribe a diuretic in addition to your ACE inhibitor or ARB. Diuretics are also called "water pills" because they help

you urinate and get rid of excess fluid in your body.

In addition to blood glucose and blood pressure control, the National Kidney Foundation recommends restricting dietary salt and protein. Your doctor may refer you to a dietitian to help you develop and follow a healthy eating plan.

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## **Hope Through Research**

In recent years, researchers have learned much about kidney disease. The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) sponsors several programs aimed at understanding kidney failure and finding treatments to stop its progression.

NIDDK's Division of Kidney, Urologic, and Hematologic Diseases (DKUHD) supports basic research into normal kidney function and the diseases that impair normal function at the cellular and molecular levels, including diabetes, high blood pressure, glomerulonephritis, and other diseases marked by proteinuria.

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## **Points to Remember**

- Proteinuria is a condition in which urine contains an abnormal amount of protein.
- The term albuminuria is also often used because some tests measure this protein specifically and it is the major type of protein in the urine.
- Proteinuria may be a sign that your kidneys are damaged and that you are at risk for end-stage renal disease.
- Several health organizations recommend that people be regularly checked for proteinuria so that kidney disease can be detected and treated before it progresses.
- Groups at risk for proteinuria and kidney failure include African Americans, American Indians, Hispanic/Latinos, Pacific Islander Americans, people who have diabetes or hypertension, and people who have a family history of kidney disease.
- You may have proteinuria without noticing any signs or symptoms. Testing is the only way to find out how much protein you have in your urine.
- If you have diabetes or hypertension, or both, the first goal of treatment will be to control your blood glucose and blood pressure.

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## **For More Information**

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